INTRA-VITAM STAINING OF TUBERCULOUS GUINEA-PIGS WITH FAT-SOLUBLE DYES.*

STUDIES ON THE BIOCHEMISTRY AND CHEMOTHERAPY OF TUBERCULOSIS. II.

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HISTORICAL.

Several experimenters have investigated the power of fat stains, especially sudan III, to stain tissues within the living animal. A review of much of this work is furnished by Riddle,[1] who developed the important fact that this dye, when fed to laying hens, is deposited in the yolk within a short time. He also found that sudan III is almost, but not altogether, without harmful effects, there being observed certain defects in the feathers and a lowered resistance to starvation, which he was inclined to ascribe to a relative lack of availability of the stained fat for the metabolism of the animal. However, this degree of toxicity must be low, since it was found by the Gages[2] that eggs dyed in this way will hatch out normal chicks with stained body fats. Birds seem to be especially favorable subjects for this sort of work, depositing the dye abundantly and rapidly, though it can be taken up also by the fat tissues of mammals and reptiles. It seems that the dye accompanies the fat in which it is given or with which it is absorbed, so that it is deposited in fat depots and similar places that are lying on fat, but not in fatty tissues which are not lying on fat, e.g., the fat tissues of wasting animals, or in lipoid-rich tissues such as the central nervous system. Neither does the dye seem to enter the normal fatty lipoid constituents of the parenchymatous cells of the viscera. When the fat dye is given to guinea-pigs by itself, not dissolved in fat, it does not color even the depot fats appreciably; likewise scarlet R., when given in vaseline or lanolin, substances unavailable for metabolism, does not become deposited in the tissues (Jacobsthal[3]). According to Mendel and Daniels[4] the dye is excreted in the bile, in which it is very soluble, which probably explains the absence of the dye in livers with fatty degeneration experimentally produced by phlorhizin or phosphorus in animals whose fat has been stained with fat dyes (Jacobsthal, Mendel, and Daniels). Jacobsthal states that a certain amount of staining may occur in fat in the liver cells if the degeneration is produced by hunger and exposure, and very large doses of stained fat are given; and Mendel and Daniels found that when very large amounts of deeply stained fat are given there may be some staining in the liver. Schmorl[5] also found some staining of fat in necrotic renal cells.

From the above papers it seems evident that there is no marked tendency for sudan III deposited in the depot fats, or present in the food fats, to enter the fat droplets present in degenerated parenchyma cells, at least in the liver and kidney; but this

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[1] *Jour. Exper. Zoöl.*, 1910, 8, p. 164.

[2] *Anat. Record*, 1909, 3, p. 203.

[3] *Verhandl. deutsch. path. Gesellsch.*, 1909, 13, p. 380.

[4] *Proc. Soc. Exper. Biol.*, 1911, 8, p. 126.

[5] In discussion following Jacobsthal's paper.

negative result may be explained by the fact that in these two tissues we have the places of excretion of the dye, which may interfere with its storage, especially in the liver where we have an excess of bile in which the fat dyes are very soluble.

Other fat dyes seem to have received relatively little consideration as *intra-vitam* stains. Jacobsthal worked chiefly with scarlet R., which seems to behave much the same as sudan III. He failed to get results by intravenous injection of Nile blue sulfate, or by feeding indophenol.

Alkanna, which is a vegetable dye and not a synthetic product like most of the fat dyes, seems to behave much like sudan III when used as an *intra-vitam* stain, according to the results obtained by Hofbauer.[1] Mendel and Daniels[2] have recently described experiments with Biebrich scarlet, which gives results comparable to those obtained with sudan III; with indophenol-blue which gave negative results, attributed to the reduction of this dye in the body; with "oil-soluble green" and annatto which gave entirely negative results.

There seem to be but few recorded instances of the feeding of fat dyes to man, one being a case of chyluria studied by Franz and Stejskal.[3] They administered five grams of olive oil "mit Sudan III intensiv roth gefärbt," and found that the dye appeared in the urine. Hofbauer[4] gave sudan III in capsules to a woman twenty hours previous to a Cesarean section, and claims that he found the dye in both maternal and fetal blood. Davidsohn[5] records an interesting case of a child which died after being given a "carrot cure"; the body fat was found everywhere deeply stained a carrot color, and as the pigment of carrots, carotin, is known to be a fat-soluble dye this may be fairly considered an instance of specific *intra-vitam* fat staining.

As tubercles contain a certain amount of fat, associated with disintegrative changes in the cells, and especially because of the fatty character of the tubercle bacillus itself, a set of experiments was performed to ascertain to what extent fat dyes administered to living tuberculous animals might penetrate the tubercles and stain the fat therein contained; also, incidentally, to note what effect, if any, the fat dyes might have upon the progress and development of the tuberculosis. All the dyes used in these experiments were from Grübler.

EXPERIMENTAL.

FEEDING EXPERIMENTS WITH SUDAN III.

A series of guinea-pigs were fed a one per cent solution of sudan III in peanut oil (10 c.c. per kilo body weight) every second day during the period of the experiment.

Experiment 1.—Sudan III was fed for 88 days, at the end of which time the animal died of tuberculosis, having been inoculated on the 47th day after feeding was begun.

[1] *Pflügers Archiv*, 1900, 81, p. 263.

[2] *Jour. Biol. Chem.*, 1912, 12, p. 71.

[3] *Ztschr. f. Heilk.* (inn. med.), 1902, 23, p. 441.

[4] Quoted by Mendel and Daniels.

[5] In discussion following Jacobsthal's paper.

The postmortem findings showed all the fatty tissues, the subcutaneous, mesenteric, omental, testicular, etc., stained pink. The local glands at the inguinal region where the primary inoculation of the tubercle bacilli was made, were markedly enlarged but stood out distinctly unstained in comparison to the surrounding pink fat. The spleen contained numerous foci of necrosis, a few such areas were found in the lungs and the liver, but none of these appeared pink. The salivary glands were unstained. The kidneys and adrenals were unstained, but the surrounding fat was pink. The brain and cord was also unstained. The pus obtained from the inguinal glands contained numerous tubercle bacilli stainable by carbol fuchsin, but sudan III had not stained them *in vivo*. Frozen sections of the liver, kidneys, and spleen did not reveal any sudan staining.

The different tissues from this animal were dried and mixed with anhydrous Na_2SO_4 and were then extracted in a Greene extraction apparatus[1] by means of ether for five to eight hours, with the following results (++ designates an intense red color, + a red color still perceptible and unquestionable, −no red perceptible):

Lung, ++ (aspiration of dye accounts for the large amount of dye present in the lung extract in this and other experiments)	Fat at head of testes, ++
Other body fats, ++	Liver, +?
Kidney, −	Testes, −
Heart (ventricle), −	

Experiment 2.—This guinea-pig received sudan III for 241 days, at the end of which time it was killed, having been inoculated with tuberculosis on the 130th day after feeding with sudan III was begun.

The postmortem findings are practically identical with those in Experiment 1, with only slight differences in the tubercular involvement.

Experiment 3.—This guinea-pig received sudan III every other day for 216 days, and died at the end of this time of tuberculosis, having been inoculated on the 130th day after feeding was begun.

As in the first two pigs the postmortem revealed sudan III staining in all the fatty tissues, and none perceptible in the parenchymatous organs or tuberculous tissues.

Experiment 4.—This guinea-pig received sudan III every other day for 123 days, and died at the end of this time of tuberculosis, having been inoculated on the 47th day after feeding was begun.

Except for slight differences in the distribution of the tuberculosis this animal did not differ markedly from the previous pigs in the distribution of the sudan III.

Experiment 5.—A guinea-pig received sudan III for 83 days, at the end of which time it was killed, having been inoculated with tuberculosis on the 47th day after feeding with sudan III was begun.

Postmortem examination revealed the stain in all the fatty tissues as had been found in the previous pigs. The parenchymatous organs were not stained. The liver and spleen contained numerous small foci of necrosis unstained by sudan III. The inguinal glands contained a large amount of caseous material without a trace of pink color.

Experiment 6.—A control guinea-pig not inoculated with tuberculosis, received sudan III for 241 days, at the end of which time the animal was killed.

[1] *Jour. Biol. Chem.*, 1910, 7, p. 503.

Postmortem revealed a well-nourished animal in contrast to the previous five pigs, which were all emaciated and poor in body fat. All the fats of the body, subcutaneous, mesenteric, testicular, at the head of the kidney, etc., were stained pink, differing from the previous pigs only in amount of fat present. Parenchymatous organs revealed no pathological changes and were not stained. The brain and cord were negative for stain.

Experiment 7.—A guinea-pig with advanced tuberculosis was fed sudan III in oil four times in five days, at the end of which time the animal died of tuberculosis.

Autopsy revealed no pink coloration of the fats of the body and the ether extract of the liver, lung, kidneys, and adrenals, caseous glands and spleen did not contain any sudan III. (The extractions were carried out as previously described for Experiment 1.)

Experiment 8.—A second guinea-pig with advanced tuberculosis was fed sudan III in oil seven times (2 c.c. of one per cent solution) in eight days, at the end of which time the animal died of tuberculosis.

Postmortem revealed a large caseous set of left inguinal glands which were not stained, and numerous necrotic areas in the spleen, liver, and lungs. The body fats were sparse (atrophy of fat) but where small amounts were found, as in the mesentery and at the head of the testes, these were a faint brown-pink tint. The lungs contained a large amount of sudan III (aspiration of stained fat during feeding). The necrotic areas in the liver and spleen were not stained. Adrenals, kidneys, gall bladder, etc., were not stained.

Results of extraction of the various tissues by ether (as previously described for Experiment 1) were:

Lungs, ++	Liver, ++? The color of the extract was a deep brown-red and appeared to be deeply stained by sudan
Testes and fat, +?	Gall bladder and contained bile, −
Spleen, −	Kidneys, −
Adrenals, −	Caseous glands, −

Experiment 9.—A third tuberculous guinea-pig was fed sudan III in oil eight times in nine days, at the end of which time the animal died of tuberculosis.

Autopsy revealed enlarged caseous glands in the left inguinal region, and caseous and necrotic material in the upper left thigh, neither of these containing any pink stain. The fatty tissues were not pink but rather brown in appearance (atrophy). At the head of the testicles the fat appeared of brownish-pink hue. The spleen was much enlarged and composed of numerous necrotic areas. The liver was nearly all necrotic, but unstained. Lung contained numerous necrotic areas but no visible stain. Gall bladder was distended but unstained. Adrenals, kidneys, and brain were unstained. Results of ether extraction were as follows:

Lungs, +	Gall bladder and contained bile, +? a deep brown-red color
Liver, −	Kidneys, −
Brain, −	Spleen, −
Adrenals, −	Testes and fat at head of testes, −?

Experiment 10.—A fourth tuberculous guinea-pig was fed sudan III in peanut oil 22 times in 26 days, at the end of which time the animal died of tuberculosis.

At autopsy were found numerous enlarged caseous lymph glands (local and general) unstained, the liver was one mass of necrotic areas (unstained), there were large necrotic areas in the spleen which contained no dye, and the lungs also contained numerous small necrotic areas. The fats of the body were markedly atrophied, so that practically no fat was found in the ordinary fat depots and no visible stain was found.

The results of ether extraction of the tissues of this animal were as follows:

Lungs, ++	Caseous lymph glands, −
Liver, −	Kidneys, −
Spleen, −	Adrenals, −

As a result of the above feeding experiments with sudan III in oil, the following conclusions seem justified:

1. Sudan III in oil, fed to normal guinea-pigs, stains all the body fats pink but does not visibly stain the parenchymatous organs of the body.

2. Sudan III dissolved in oil, fed to normal guinea-pigs which were later inoculated with tuberculosis and the feeding continued, also stained the body fats pink, but did not stain the tuberculous lesions (necrotic areas in the liver, spleen, and caseous glands).

3. Sudan III in oil, fed to tuberculous guinea-pigs for a short period of time (4–8 feedings) previous to death resulting from the disease, did not stain the lesions or enter the body fats to any appreciable extent.

4. Sudan III dissolved in oil and fed to tuberculous guinea-pigs did not in any way influence the progress of the disease.

5. Sudan III may be fed to guinea-pigs for a long period of time (over 200 days) in quantities of 0.05 gm. every second day, without appreciably injuring the animal.

INJECTIONS OF SUDAN III.

A sterile one per cent solution of sudan III in olive oil or butter was injected into normal guinea-pigs subcutaneously and intraperitoneally.

Experiment 11.—A guinea-pig received subcutaneously four cubic centimeters of one per cent solution of sudan III in olive oil every other day, and died on the 22d day after the first injection.

Postmortem revealed large amounts of sudan III stained oil diffused through the subcutaneous tissues with numerous small subcutaneous, gangrenous areas. The peritoneal fat was unstained, but the fluid in the peritoneal cavity had an oily appearance. The amount of oil diffused through the subcutaneous tissues and the gangrene were sufficient to account for the death of the animal.

Experiment 12.—Another guinea-pig was injected subcutaneously with sudan III olive oil every second day, and died on the 20th day.

The autopsy findings were practically the same as those observed in Experiment 10.

Experiment 13.—Another guinea-pig received four cubic centimeters of one per cent solution of sudan III in olive oil intraperitoneally every other day for 60 days, and died 93 days after the injections were begun.

Autopsy revealed about 200 c.c. of oily liquid in the peritoneal cavity, the oil being deeply stained with sudan III. The fat at the head of the testes was not stained, the mesenteric fat was pink but only superficially. Fibrinous-like masses of stained material were found around the liver and under the diaphragm. The mesenteric lymph glands were markedly enlarged and contained a large amount of sudan III. The omentum was rolled up into a mass containing fat and stain. The parenchymatous organs were not visibly changed and contained no stain.

In addition to these experiments butter was substituted for olive oil and a one per cent solution of sudan III emulsion in sterile butter was injected intraperitoneally.

Experiment 14.—A guinea-pig was injected intraperitoneally every second day with one per cent solution of sudan III in butter for 16 days, at the end of which time the animal died.

At autopsy the sudanized butter was found to a slight extent in the subcutaneous tissues at the site of injection, but mainly in the peritoneal cavity. The fatty tissues in the peritoneal cavity were only superficially stained. The omentum was deeply stained. Parenchymatous organs were not stained.

Experiment 15.—A duplicate of No. 14, lived for 15 days.

At autopsy the sudanized butter was found to be infiltrated throughout the subcutaneous and peritoneal tissues. Fatty tissues (that is, the mesenteric, post-abdominal, etc.) were not perceptibly tinted. A serofibrinous exudate was found in the peritoneal cavity, which was of deep red color as a result of impregnation with stain.

Experiment 16.—A duplicate of No. 14, lived 14 days after beginning injections.

At autopsy the small intestines were adherent in many places at the site of injection, and the peritoneal cavity contained a large amount of sudanized butter. The peritoneum was tinted pink and the fatty tissues around the uterus and tubes, kidneys and in the mesentery were superficially pink stained.

As a result of the above experiments with injections of sudan III in oil, the following conclusions seem justified:

1. After the intraperitoneal injection of sudan III dissolved in oil or in sterile butter into guinea-pigs for a period of 14 to 60 days, the sudan III remains practically unabsorbed in the peritoneal cavity where it stains superficially the body fats with which it comes in contact.

2. After subcutaneous injection into guinea-pigs of sudan III

dissolved in oil, only the fats with which the injected sudanized oil comes in contact are stained, while the other body fats (peritoneal fats, etc.) remain unstained.

A saturated (70 per cent) alcoholic solution of sudan III was injected into the testicles of guinea-pigs and was found to remain at the site of injection for a period of several days. Longer periods of time will be reported later.

FEEDING EXPERIMENTS WITH SCARLET R.

In the following feeding experiments a one per cent solution of scarlet R. in peanut oil was given to guinea-pigs.

Experiment 17.—A guinea-pig was fed six cubic centimeters of a one per cent solution of scarlet R. in peanut oil every other day for 209 days, having been inoculated with tuberculosis on the 38th day after feeding was begun, and dying of tuberculosis at the end of the feeding experiment.

Autopsy revealed a double hydrothorax and multiple areas of necrosis in the lungs, liver, and spleen which were not stained by scarlet R. Scarlet R. was, however, found in all the fat tissues, mesenteric fat, along the psoas, along the tubes and around the local enlarged caseous tuberculous glands, but the glands themselves and their caseous contents were unstained. Frozen sections of the liver, kidney, adrenals, etc., revealed no stain in the parenchymatous organs.

A guinea-pig was born on the 50th day after feeding of the mother was begun, but its body fats were not stained by scarlet R. The milk of the mother did not contain any stain, either that taken from the mammary glands or that found in the young pig's stomach. The liver of the young pig was pale and fatty, containing a large amount of unstained fat which was stainable histologically by sudan III.

Experiment 18.—A duplicate to the previous experiment was carried out, feeding scarlet R. for 166 days, the guinea-pig having been inoculated with tuberculosis on the 38th day after feeding was begun, and dying of tuberculosis at the end of the feeding experiment.

Postmortem revealed all the fatty tissues stained a dark pink (subcutaneous fatty tissues, mesenteric fat, along tubes, at the head of the kidneys, at spleen pedicle, etc.). Both lungs were solid and hard (tuberculous pneumonia) and contained a large amount of scarlet R. There was a general enlargement of lymph glands and the primary inguinal glands were large and contained a large amount of unstained caseous material. The brain and mammary glands were not stained. Ether extraction of the tissues, carried out as described in Experiment 1, resulted as follows:

Lung, ++
Spleen, +? A very faint pink tint
Kidneys, −.
Liver, +?
Fats around uterus and tubes, ++
Adrenals, −? (There may have been a bare trace of pink here, but two observers called it negative)

A pig was born to this guinea-pig on the 85th day after feeding was begun. Autopsy revealed no scarlet R. in its fat tissues, but the liver was pale and fatty, so

that histological staining with sudan III revealed a large amount of stainable fat. The milk in the stomach of this young pig was unstained, as was also that obtained from the gland of the mother pig.

Experiment 19.—A control guinea-pig, not inoculated with tuberculosis, was fed scarlet R. in peanut oil for 197 days, at which time autopsy revealed all the fat tissue stained a deep pink. The liver was normal in appearance and contained no visible stain macroscopically or microscopically on frozen section. The kidneys, lungs, spleen, brain, and cord contained no stain and were normal. There was no atrophy of body fats as was noted in the tuberculous pigs.

A guinea-pig was born 47 days after scarlet R. feeding was begun. At autopsy, shortly after birth, the body fats contained no stain, nor did the parenchymatous organs. The mesenteric lacteals stood out white and unstained in this pig. The milk from the mother and that found in the stomach of the young pig was unstained.

As a result of the above feeding experiments with scarlet R. in guinea-pigs the following conclusions seem justified:

1. Scarlet R. fed to guinea-pigs, normal and tuberculous, for a long period of time (166–209 days), stains all the body fats pink, but does not stain the parenchymatous organs.

2. Scarlet R. in oil fed to tuberculous guinea-pigs previous to and during the disease does not stain the tuberculous lesion or noticeably affect the progress of the disease.

3. Scarlet R. in oil fed to guinea-pigs for a long period of time (47–85 days), does not stain any of the tissues of the young or pass into the milk of the adult guinea-pig.

4. The feeding to the mother of a large amount of oil stained with scarlet R. produces a fatty liver in the unborn young.

EXPERIMENTS WITH INJECTIONS OF NILE BLUE SULFATE.

This is a water-soluble fat dye which possesses the added advantage of staining the neutral fats and fatty acids differently from one another.[1]

Subcutaneous and intraperitoneal injections in physiological salt solution were made in the following experiments:

Experiment 20.—A guinea-pig was injected subcutaneously on alternate days with three cubic centimeters of two per cent Nile blue sulfate in physiological salt solution in three different places; a week after the first injection the skin and surrounding tissues became necrotic and sloughed out.

Experiment 21.—A second guinea-pig, duplicate of 20, developed the same necrosis, and sloughing of the skin.

[1] For literature on Nile blue sulfate see J. Lorrain Smith, *Jour. Path. and Bact.*, 1907, 12, p. 1; and Holthusen, *Zieglers Beitr.*, 1910, 49, p. 595.

Experiment 22.—A guinea-pig was given intraperitoneally three injections of three cubic centimeters two per cent Nile blue sulfate in physiological salt solution on alternate days, and was found dead on the eighth day.

Postmortem revealed a large amount of fibrinous exudate in the peritoneal cavity, which contained a blue stained fluid. The abdominal viscera were speckled with particles of stain, but not uniformly stained. The spleen and kidneys were not stained, but simply covered with a fibrinous exudate containing blue particles; the liver was also unstained. The lymphatic vessels of the anterior thoracic wall were slightly blue tinted. Heart, lungs, adrenals, brain, and cord were unstained.

Experiment 23.—A guinea-pig treated exactly as 22 was also dead in eight days. The stain was mainly found in the fibrinous exudate in the peritoneal cavity, the lymphatics in the anterior thoracic wall not being stained in this animal.

FEEDING EXPERIMENTS WITH NILE BLUE SULFATE.

Feeding experiments with Nile blue sulfate were carried out, using a one per cent emulsion in peanut oil. Attempts were made to feed the dye in aqueous solution, but without success because the guinea-pigs reject water solutions of the dyes.

Experiment 24.—A guinea-pig was given on alternate days five cubic centimeters of one per cent solution of Nile blue sulfate in peanut oil for 151 days, having been inoculated with tuberculosis on the 38th day after feeding was begun. At the end of the feeding period, the stain was not found anywhere in the body of the animal, but the animal had numerous large necrotic areas in the liver and spleen, and the lymph glands of the body were enlarged, the left inguinal glands containing a large amount of caseous material unstained. After a general rapid inspection of the animal a forced aortic injection of one per cent Nile blue sulfate in 0.9 per cent NaCl solution containing a small amount of hydrogen peroxid was made. The stain rapidly passed through the tissues, but did not stain the necrotic areas or the fats to any extent. In the liver and spleen a few nondescript nuclei were stained, otherwise the experiment was negative.

Experiment 25.—A guinea-pig (duplicate of 24) was fed one per cent solution of Nile blue sulfate in peanut oil for 149 days, having been inoculated with tuberculosis on the 38th day and dying of tuberculosis at the end of the feeding period.

Postmortem revealed numerous necrotic areas in the spleen and liver unstained. The local inguinal glands were caseous and unstained. The body fat was atrophied but unstained. The kidneys, adrenals, lungs, etc., were unstained. The tissues treated with H_2O_2 did not take on any blue color.

Experiment 26.—A normal guinea-pig was fed one per cent solution of Nile blue sulfate in peanut oil for 221 days, at which time it was still in good health. Killed with ether.

Autopsy revealed no stain (macroscopically or microscopically) in the fat tissues of the body or in any of the parenchymatous organs.

A guinea-pig was born 103 days after feeding was begun but contained no stain, either in the fat tissues or parenchymatous organs. The milk from the mother and that found in the young pig's stomach was not stained.

Another guinea-pig was born 187 days after beginning feeding.

Postmortem revealed no stain anywhere in the young pig. The milk again was negative for stain. The liver of the young pig was pale and fatty. (Sudan III staining revealed a large amount of fat in the liver.)

A second pig born 187 days after feeding began was allowed to live eight days, at the end of which time no stain was found in the body. The liver, however, was still pale, but not so much so as that in the previous young pig.

As a result of the above experiments with Nile blue sulfate the following conclusions seem justified:

1. Nile blue sulfate in two per cent solution in 0.9 per cent NaCl is toxic when injected intraperitoneally, causing a marked serofibrinous peritoneal exudate, and death after three injections of three cubic centimeters on alternate days. Subcutaneously injected, it causes necrosis of the tissues and sloughing at the site of injection, the stain itself not staining any of the tissues but rather remaining at the site of injection.

2. Although highly toxic when injected, Nile blue sulfate seems to have no toxic effect when fed in oil for long periods of time.

3. Feeding of Nile blue sulfate in oil emulsion did not lead to staining of the fats or parenchymatous organs of the guinea-pig.

4. Feeding Nile blue sulfate in oil emulsion to guinea-pigs infected with tuberculosis after feeding had begun did not result in staining the tuberculous lesions (caseous lymph glands and necrotic areas in the spleen and liver) or visibly influence the progress of the disease.

FEEDING EXPERIMENTS WITH SUDAN YELLOW (META-DIOXYAZOBENZOL).

Four guinea-pigs were fed a one per cent solution of sudan yellow in peanut oil, two of these were inoculated with tuberculosis and two remained non-infected throughout the entire period of the experiment.

Experiment 27.—A normal guinea-pig was fed sudan yellow on alternate days for 144 days, at the end of which time postmortem revealed a normal liver (not fatty), lungs, kidneys, adrenals, brain, etc., containing no yellow stain visible macroscopically or microscopically. The fats of the body were not more yellow than found in normal unfed animals. The urine in the bladder was a distinct yellow, however, whereas frozen sections of the kidney did not contain any of the yellow dye.

Experiment 28.—A second normal guinea-pig was fed sudan yellow for 104 days and the postmortem findings were the same as those observed in the previous pig. No macroscopic stain was observed in any of the tissues of the animal, but the bladder was filled with a deep yellow urine, and frozen sections of the kidney appeared to contain a little yellow pigment, especially in the glomeruli.

Experiment 29.—A guinea-pig was fed one per cent solution of sudan yellow in peanut oil every other day for 97 days, having been inoculated with tuberculosis on the 33d day of the feeding.

Autopsy at the end of the feeding period revealed necrotic areas in the liver, spleen, and a few in the kidneys. The necrotic areas in the liver and spleen were of a greenish yellow color, but the yellow was not due to the dye as the same yellowish coloration had been noticed in animals with tuberculosis not receiving any dyes, and as shaking out the tissue with ether revealed no yellow coloration of the ether layer beyond that ordinarily obtained from liver tissue. The local caseous inguinal glands were unstained. The fat tissues were atrophied but contained no dye. The urine in the bladder was a deep yellow color, but the kidney was not stained.

A guinea-pig was born on the 88th day after feeding was begun, but it contained none of the yellow dye. The milk of the mother pig was not stained yellow.

Experiment 30.—A second guinea-pig was fed sudan yellow as in preceding experiment for 145 days, having been inoculated with tuberculosis on the 33d day after feeding was begun.

Autopsy at the end of the feeding period revealed enlarged caseous local inguinal glands (unstained) and enlargement of the lymph glands generally (unstained), numerous necrotic areas in the liver of greenish yellow color, the spleen was much enlarged and contained large, white necrotic areas. The fat tissues of the body (at the head of the testes, posterior abdominal, and at the back of the neck) were stained a faint pale yellow color. The brain and cord were unstained. There was no urine in the bladder and the bladder itself was not stained yellow.

The above experiments in which sudan yellow was fed to normal and tuberculous guinea-pigs seem to justify the following conclusions:

1. Sudan yellow fed to normal and tuberculous guinea-pigs does not stain the organs of that animal nor to an appreciable degree, the body fats within 145 days, but it does color the urine yellow.

2. Sudan yellow fed to an adult guinea-pig does not color the milk of the mother or the young guinea-pig, even when fed for a long period of time (88 days).

3. Sudan yellow fed to guinea-pigs infected with tuberculosis after feeding was begun does not enter the tuberculous lesions (caseous lymph glands or necrotic areas in the liver and spleen).

FEEDING EXPERIMENTS WITH SUDAN BROWN (α-OXYAZONAPHTHALIN).

Normal and tuberculous guinea-pigs were fed sudan brown in peanut oil for long periods of time to see whether *intra-vitam* staining with this fat stain was possible.

Experiment 31.—A normal guinea-pig was fed four to five cubic centimeters of a one per cent solution of sudan brown in peanut oil on alternate days for 144 days, at the end of which time it was killed. Postmortem examination revealed a faint brown

tint of the fat tissues of the body, but no macroscopic or microscopic staining of any parenchymatous organs. The liver, spleen, kidneys, adrenals, and brain were normal and unstained. Bladder contained unstained urine. In the uterus were found two half-developed embryos, unstained. The placentas were unstained.

A guinea-pig, born 94 days after feeding was begun, contained no stain, either in the fat tissues or in the parenchymatous tissues. The milk of the adult guinea-pig was unstained.

Experiment 32.—A guinea-pig was fed four to five cubic centimeters of a one per cent solution of sudan brown in peanut oil on alternate days for 115 days, having been inoculated with tuberculosis on the 33d day after feeding was begun, and dying of tuberculosis at the end of the feeding period.

Autopsy revealed practically no visible stain in the fat tissues. The local inguinal glands were enlarged and caseous, but were not stained. The liver and spleen contained numerous large necrotic areas which were pale yellow and white (unstained). The adrenals, kidneys, brain, etc., were unstained macroscopically and microscopically.

Experiment 33.—A second guinea-pig was fed sudan brown on alternate days for 145 days, having been inoculated with tuberculosis on the 33d day. At the end of the feeding period autopsy revealed a faint brown tint in the fat tissues of the body (especially at the back of the neck and around the tubes). The liver, spleen, and lung contained numerous white and yellow necrotic areas, unstained. The local inguinal glands were enlarged and caseous, but unstained. The kidneys, adrenals, bladder, urine, brain, and cord were unstained.

As a result of the above feeding experiments with sudan brown, the following conclusions seem justified:

1. Sudan brown when fed, stains the body fats of normal and tuberculous guinea-pigs only slightly, if at all, within 145 days. It does not stain any of the parenchymatous organs.

2. In tuberculous animals sudan brown, fed for a long period of time, does not stain the tuberculous lesions (caseating lymph glands and necrotic areas in the liver, spleen, or lungs).

FEEDING EXPERIMENTS WITH EOSIN A.

A series of four guinea-pigs were fed Eosin A, water-soluble, in suspension in peanut oil for periods of 100 to 133 days. Two of the pigs remained normal throughout the feeding period, and two of them were inoculated with tuberculosis on the 21st day after feeding was begun. The results obtained with these animals were entirely negative, the dye was not found in any of the parenchymatous organs or tissues, so that absorption apparently had not taken place by this method of administration. The tuberculous lesions (local caseous lymph glands, necrotic areas in the lungs, liver, and spleen) also were unstained.

DISCUSSION OF RESULTS.

These experiments carry over to the lesions of tuberculosis the results obtained by the biologists who have investigated the behavior of sudan III and other fat stains under normal and some

pathological conditions. Their results indicate that the fat dye which enters the body dissolved in fat remains either entirely or chiefly with this same food fat, being deposited with it if the food fat is deposited, but not leaving the food fat to enter either stored fat or the intracellular fats and lipoids of active tissues. *The fats of tubercles never show any of the administered fat dyes*, no matter whether the tubercles formed before or after the animal was saturated with the dye. Therefore, it seems probable that the fats microscopically visible or chemically demonstrable in tubercles, are derived chiefly or solely from the existing fats and lipoids of the disintegrated cells, not by deposition from the fats in the blood, a view entirely in harmony with the histological evidence. Attention may also be called to the absence of any visible staining of the tubercle bacilli within the lesions by the administered fat dyes. This result is to be expected in view of the very limited extent to which tubercle bacilli are stainable with fat dyes even *in vitro*, as shown by other experiments performed in this laboratory, which will soon be reported.

The fat dyes seem to be almost entirely innocuous to guinea-pigs, when given by mouth, as observed by other experimenters. In view of the striking effect of fat dyes upon cell proliferation as shown by B. Fischer, this apparent inertness of the same dyes when fed to animals is rather surprising. Even with tuberculous animals the thorough saturation of the animal with the dye has not seemed to lower the resistance to the disease. Female guinea-pigs, thoroughly saturated with these dyes, give birth to sound offspring which show on autopsy no abnormalities other than a fatty liver. The observation of the Gages that the fat dyes do not pass through the placenta to the fetus is confirmed by these experiments, as also by Mendel and Daniels. This fact would seem to be of great importance in general biology, for it seems to indicate that the fats of the fetus are not derived as such from the mother but are formed, either partly or wholly, in the body of the fetus itself. In contrast with this is the fact that in birds and reptiles the fat stain enters the ovum very quickly and abundantly, and during development passes into the fats of the embryo. It is difficult to believe that between birds and mammals there exists so

fundamental a difference in developmental chemistry as these observations imply, and more extensive observations along this line are needed.

The failure of fat dyes to enter brain and cord, as observed by others, is corroborated and extended to other dyes. Jacobsthal says that scarlet R. enters the adrenals, but no positive results were obtained in the animals of this series. Neither was any staining of the milk of lactating female guinea-pigs ever observed, although described in the rat by the Gages; and in cats, rats, goats and to a very slight extent in the guinea-pig by Mendel and Daniels. The reason for these discrepancies is not apparent.

SUMMARY.

Fat dyes administered under various conditions to guinea-pigs with tuberculosis never were found within the tubercles. This is in favor of the view that the fats of the tubercle are derived from the intracellular fats of the tissues forming the tubercle, and not from food fats or transported depot fats.

Tubercle bacilli within tubercles in such animals do not become stained with the fat stains.

Saturation of the animal with common fat dyes has no noticeable influence on the course of a tuberculous infection in the guinea-pig.

The fat stains employed in these experiments (sudan III, scarlet R., sudan yellow, sudan brown, Nile blue sulfate) were never found to stain any but depot fats, not being present in the parenchymatous cells whether normal or diseased. They caused no apparent harm to the guinea-pigs even after being fed to them over 200 days.

Subcutaneous or intraperitoneal injection of fat dyes dissolved in fats and oils is much less effective in staining the depot fats, than is feeding the stained fats.

Nile blue sulfate is toxic when given by injection, and when fed it did not cause staining of the fat tissues. Sudan yellow also failed to stain the fat tissues, and is excreted by the kidneys. Sudan brown has little or no effect on the fats. Scarlet R. gave a more intense coloration than sudan III but in general the effects are about the same.

Fat dyes do not pass through the guinea-pig placenta to the

fetus, or at least the fat of embryo pigs from stained mothers is not stained (corroborating the results of the Gages, Mendel, and Daniels). The embryos often show extremely fatty but unstained livers. Fat dyes were never observed to pass into the milk of lactating guinea-pigs, although positive results have been obtained by others.